

Nutrient (N and P) dynamics of the invasive macroalga *Gracilaria vermiculophylla*
nutrient uptake kinetics and nutrient release through decomposition

Pedersen, Morten Foldager; Lørup Johnsen, Kathrine

Published in:
Marine Biology

DOI:
[10.1007/s00227-017-3197-7](https://doi.org/10.1007/s00227-017-3197-7)

Publication date:
2017

Document Version
Peer reviewed version

Citation for published version (APA):
Pedersen, M. F., & Lørup Johnsen, K. (2017). Nutrient (N and P) dynamics of the invasive macroalga *Gracilaria vermiculophylla*: nutrient uptake kinetics and nutrient release through decomposition. *Marine Biology*, 164, 172.
<https://doi.org/10.1007/s00227-017-3197-7>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the public portal.

Take down policy

If you believe that this document breaches copyright please contact rucforsk@kb.dk providing details, and we will remove access to the work immediately and investigate your claim.

Nutrient (N and P) dynamics of the invasive macroalga *Gracilaria vermiculophylla*: Nutrient uptake kinetics and nutrient release through decomposition

Morten F. Pedersen* and Kathrine L. Johnsen

Department of Science and Environment (DSE)
Roskilde University, Box 260, DK-4000 Roskilde, Denmark.

* Corresponding author: Morten Foldager Pedersen, email: mfp@ruc.dk, phone: +45 46742088.

Acknowledgments

We thank technician Anne B. Faarborg for assistance in the laboratory and two anonymous referees for valuable comments to an earlier version of the manuscript.

Compliance with Ethical Standards:

Funding: This study was supported by grant no. 272-08-0577 (MARINVA) from the Danish Council for Independent Research.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

Abstract

The invasive alga *Gracilaria vermiculophylla* was introduced to Europe two decades ago and has since become dominant in many shallow estuaries. *G. vermiculophylla* is a relatively fast-growing alga that thrives well at low nutrient availability in summer, suggesting that it uses nutrients efficiently, which might give it a competitive advantage over many native species. We studied therefore the nutrient dynamics of *G. vermiculophylla* and compared it to those of a range of native macroalgal species. Nutrient uptake rates (NH_4^+ , NO_3^- and PO_4^{3-}) were compared to growth related requirements and we found that *G. vermiculophylla* needs relatively high nutrient concentrations to sustain fast and non-limited growth. This compares to the nutrient dynamics of many fast-growing algae and we found thus no indication that *G. vermiculophylla* should have any particularly advantage relative to other, sympatric species. The nutrient storage capacity of *G. vermiculophylla* was, in contrast, relatively large and comparable to that of more slow-growing algae, which, when combined with the low nutrient uptake experienced in summer, could explain how *G. vermiculophylla* can sustain non-nutrient limited growth through most of the growth season. The biomass of *G. vermiculophylla* can be massive and estimates showed that gross nutrient uptake could exceed the amount of nutrients received from land. The turnover of *Gracilaria* biomass is however fast and nutrients bound in the resulting detritus are quickly mineralized during decomposition, which is especially important during late summer when water temperatures are high. Invasion and subsequent dominance by *G. vermiculophylla* may thus affect local nutrient cycling significantly.

Key words: Nitrate, ammonium, phosphate, uptake kinetics, mineralization, seaweed.

Introduction

The red alga *Gracilaria vermiculophylla* originates from NE Asia (Tseng and Xia 1999), but was introduced to North America and Europa with imported oysters intended for aquaculture in the 1990's (Mollet et al. 1998). *G. vermiculophylla* is invasive (Nyberg et al. 2009) and has spread along the Atlantic coast of Europe and is now found from Venice lagoon in the Mediterranean to southern Norway in Scandinavia (Thomsen et al. 2007; Sfriso et al. 2012). *G. vermiculophylla* was first observed in Danish waters in 2003 and is now common in many estuaries of the western Baltic Sea (Thomsen et al. 2007; Weinberger et al. 2008). *G. vermiculophylla* is mostly abundant in sheltered, soft-bottom areas where it may become dominant and sometimes replaces previously common seaweeds such as *Ulva* sp. (Nejrup and Pedersen 2010) or *Fucus vesiculosus* (Weinberger et al. 2008). *G. vermiculophylla* is considered an ecosystem engineer (Wallentinus and Nyberg 2007; Byers et al. 2012) and where abundant it may not only affect the composition of the algal assemblage, but also affect local biogeochemistry, nutrient cycling including the transfer of nutrients between abiotic and biotic components (Tyler and McGlathery 2006; Hardison et al. 2010; Gulbransen & McGlathery 2013) and alter trophic relations in the food web (Wallentinus and Nyberg 2007).

Gracilaria vermiculophylla has a number of traits that are typical for invasive algae; it is relatively fast-growing ($22\% \text{ d}^{-1}$; Raikar et al. 2001), recruits from both spores and fragments (Rueness 2005; Thomsen et al. 2007; Nyberg et al. 2009), it is tolerant to desiccation and extreme levels of light, temperature and salinity (Yokoya et al. 1999; Raikar et al. 2001; Nejrup and Pedersen 2012; Nejrup et al. 2013). Recent studies have further shown that *G. vermiculophylla* is avoided by many native herbivores within its invaded range (Thomsen and McGlathery 2007; Weinberger et al. 2008; Nejrup et al. 2012; Hammann et al. 2013) due to inducible chemical defenses (Nylund et al. 2011; Hammann et al. 2016).

Low nutrient availability (permanently or periodically) restricts growth of fast-

growing species more than that of more slow-growing species because the latter are better adapted to cope with low nutrient availability (Pedersen and Borum 1996; Pedersen and Borum 1997; Pedersen et al. 2010) and nutrient richness may therefore affect species composition of algal assemblages (Bokn et al. 2003; Karez et al. 2004; Kraufvelin et al. 2006; Kraufvelin et al. 2010). *G. vermiculophylla* is relatively fast growing and should therefore have high nutrient demands per unit biomass and time and be susceptible to nutrient limitation during late spring and summer where insolation and water temperature is high enough to support rapid growth, but where the availability of nutrients is low. Nejrup and Pedersen (2010) studied seasonal variations in biomass and growth of *G. vermiculophylla* in two Danish estuaries with low nutrient availability in summer, but were unable to detect any significant increase in growth following experimental nutrient enrichment. This result indicates that *G. vermiculophylla* has the capacity to acquire dissolved nutrients efficiently even when these are present at low concentrations or, that it is able to sustain growth in summer by using internal nutrient reserves obtained during winter and early spring where nutrient availability is high. Both strategies are comparable to those of more slow-growing species that can sustain near maximum growth rates during extended periods of low nutrient availability (Pedersen and Borum 1996; Pedersen and Borum 1997; Pedersen et al. 2010).

Fast growth combined with an efficient uptake capacity (relative to its demands) and/or high nutrient storage capacity may leave *G. vermiculophylla* competitively superior under low nutrient availability, which could add to explain its recent success in European and north American estuarine waters. Few studies have investigated the nutrient dynamics of *G. vermiculophylla* and most of these have only assessed one or a few aspects of the nutrient dynamics, i.e. either uptake kinetics (Tyler et al. 2005; Tyler and McGlathery 2006; Abreu et al. 2011) or the role of *in situ* nutrient limitation (e.g. Thomsen and McGlathery 2007; Nejrup and Pedersen 2010). No studies have yet evaluated the full set of dynamics including uptake

kinetics, requirements for growth and the role of stored nutrients.

Seaweeds may acquire and temporarily immobilize a large proportion of the nutrients received from land, especially in shallow estuaries and coastal lagoons where algal biomass can be substantial (Tyler and McGlathery 2003; Pedersen et al. 2004). Nutrients incorporated into macroalgal biomass become temporarily unavailable for other primary producers until they are released through grazing or decomposition. The turnover rate of algal biomass and, hence, the release of nutrients, differs systematically among seaweeds with different life strategies; slow-growing macroalgae tend generally to be grazed less and decompose more slowly than fast-growing species (e.g. Buchsbaum et al. 1991; Enriquez et al. 1993; Banta et al. 2004; Conover et al. 2016). Algal assemblages dominated by fast-growing and bloom-forming macroalgae do therefore have a faster and more variable turnover of nutrients than those dominated by slow-growing, perennial macrophytes (Duarte and Cebrián 1996; Banta et al. 2004) and release of nutrients from dense populations of opportunistic seaweeds may periodically exceed land-derived inputs and the efflux of nutrients from sediments (Tyler et al. 2003). Grazing on *G. vermiculophylla* is insignificant (e.g. Weinberger et al. 2008; Nejrup and Pedersen 2010) so most of the nutrients contained in the biomass must consequently be released through decomposition. Invasion and subsequent dominance by *G. vermiculophylla* may thus potentially influence nutrient dynamics at the ecosystem level if the decomposition rate of *G. vermiculophylla* differs markedly from the species it has replaced.

The aim of this study was 2-fold. The major aim was to evaluate all aspects of the nutrient dynamics of *G. vermiculophylla* and to compare it to those of other common, indigenous algal species to assess whether *G. vermiculophylla* uses nutrients more efficiently than the algae it is potentially competing with. We wanted next to study the release of major nutrients (N and P) from decomposing *G. vermiculophylla* and compare it to that of other common, indigenous algal species to assess how *G. vermiculophylla* may affect the turn over

of nutrients in estuarine systems once it has become dominant. We measured nutrient uptake kinetics for ammonium (NH_4^+), nitrate (NO_3^-) and phosphate (PO_4^{3-}) and compared those to growth related N and P requirements, the latter being determined from culturing algae under a range of nutrient concentrations. These data were combined and used to model how growth relates to nutrient availability and additionally, used to estimate the storage capacity for N and P in *G. vermiculophylla*. We conducted finally a series of decomposition experiments to evaluate how fast N and P bound in biomass was released from dead and decaying *G. vermiculophylla* under different temperature regimes

Methods

Gracilaria vermiculophylla was collected at Fyns Hoved, Denmark (55° 36.9' N, 10° 36.7' E) in October 2013. The algae were cleaned and transported to the laboratory where they were kept in 80 L storage tanks until being used in the experiments. The storage tanks and the experimental chambers used for the uptake and growth experiments were kept at constant temperature (15°C) and salinity 25 (PSU) and were illuminated by lamps equipped with halogen spots (OSRAM 12V, 35W) providing a light intensity of ca. 90 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ PAR in a 16:8 hr light:dark cycle. The chosen temperature and salinity is optimal for *G. vermiculophylla* (Nejrup and Pedersen 2012) while the light intensity is sufficient to saturate growth of this species (ca. 60 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$; Nejrup et al. 2013). The initial N and P content in the algae were 1.7 % DW and 0.19 % DW, respectively.

Nutrient uptake kinetics. Uptake kinetics for NH_4^+ , NO_3^- and PO_4^{3-} were determined in three separate experiments using a combination of the multiple-flask and the depletion methods as described by Harrison et al. (1989) and Pedersen (1994). Eight PVC-beakers (Vol = 1.6 L) were filled with a known volume (1.0-1.4 L) of seawater (salinity 25) with different added concentrations of the nutrient species in question (NO_3^- range: 15-76 μM ; NH_4^+ range: 1-76

μM ; PO_4^{3-} range: 1-14 μM). Each beaker was bubbled with air to ensure circulation and reduce the thickness of boundary layers. Measurements of nutrient uptake were initiated by adding an algal sample (5-10 g FW) to each beaker where it was attached to a PVC-net to keep it submerged during the incubation. Three replicate water samples (each 5.0 mL) were taken from each beaker during the first hour of the incubation (at time = 0, 15, 30, 45 and 60 minutes) and then subsequently at every 30 min for the remaining part of the experiment. Water samples were immediately frozen at -20°C for later analysis of nutrients. The incubations lasted for 120 – 300 minutes and all algae were subsequently harvested and dried to constant dry weight (DW) at 85°C and weighted. Concentrations of NO_3^- in the water samples were analyzed using a Lachat (QuickChem FIA+ 8000 Series) autoanalyzer while concentrations of NH_4^+ and PO_4^{3-} were analyzed manually; the concentration of NH_4^+ was analyzed using the salicylate-hypochlorite method (Bower and Holm-Hansen 1980) while that of PO_4^{3-} was analyzed spectrophotometrically following Strickland and Parsons (1968).

Biomass specific nutrient uptake rates (V) were estimated from changes in substrate concentration (S) over the course of the experiment:

$$V = \frac{(S_0 \times Vol_0) - (S_T \times Vol_T)}{t \times B} \quad (\text{Eq. 1})$$

where V is the uptake rate (in $\mu\text{mol g}^{-1} \text{DW h}^{-1}$), S_0 and Vol_0 are the substrate concentration (in μM) and volume (in L) at the beginning of a time interval, while S_T and Vol_T are the substrate concentration and volume at the end of a time interval. t is the time elapsed between two successive samplings and B is the DW biomass. Uptake rates determined on algae from different beakers, but obtained during identical time intervals, were plotted against the mean substrate concentrations obtained in each beaker during that specific time interval. The Michaelis-Menten function (Eq. 2) was fitted to data by least square non-linear regression using SYSTAT v. 13:

$$V = \frac{V_{max} \times S}{K_m + S} \quad (\text{Eq. 2})$$

where V is the uptake rate (in $\mu\text{mol g}^{-1} \text{DW h}^{-1}$), V_{\max} is the maximum uptake rate, K_m is the half-saturation constant (in μM) and S is the substrate concentration (in μM). Uptake rates of NH_4^+ and PO_4^{3-} were initially enhanced when the algae were exposed to nutrients (figure 1) while no such transiently enhanced uptake was observed in the case of NO_3^- . Parameter estimates of V_{\max} and K_m (table 1) were therefore represented by 2 sets of uptake kinetics: (1) one representing transiently enhanced uptake rates (i.e. surge uptake), measured over the initial 30 minutes after exposure to nutrients and, (2) one representing rates obtained after 90 minutes in the case of NH_4^+ and later than 30 minutes after exposure in the case of NO_3^- and PO_4^{3-} (hereafter called assimilation).

Nutrient requirements. Algae were first pre-conditioned for 4-6 weeks in 10 aquaria (volume = 20 L) receiving different quantities of dissolved N or P, to obtain specimens with different tissue nutrient concentrations. Nutrients were added from stock-solutions of NH_4NO_3 and KH_2PO_3 . Algae receiving different levels of NH_4NO_3 received KH_2PO_3 in excess while algae receiving different levels of KH_2PO_3 received NH_4NO_3 in excess to ensure that only one nutrient was limiting at the time. The aquaria were exposed to low light ($40 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR in a 16:8 hr light:dark cycle) using shade screens to ensure slow growth, which eased the accumulation of nutrients in the tissues. Tissue N-concentrations in algae intended for the N-growth experiment ranged from 1.0 to 3.7% N of DW at the end of the pre-condition period while the P-content in these algae averaged 0.23 % of DW. Final tissue P-concentrations in algae intended for the P-growth experiment ranged from 0.04 to 0.27 % of DW while the N-content in these algae averaged 2.4 % of DW .

Growth measurements were subsequently conducted in PVC-beakers (volume = 1.6L), which were filled with GF/C-filtered seawater (salinity 25) that had been stripped for inorganic nutrients by letting *Ulva lactuca* grow in it for 2-3 days prior to use. The beakers were placed under saturating light ($90 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR) and each beaker was bubbled with

atmospheric air to create circulation and ensure exchange of O₂ and CO₂. The initial FW biomass was determined on all pre-conditioned specimens prior to the growth measurements and the DW:FW ratio (and initial tissue N or P concentrations) was determined on a number of sacrificed subsamples after drying them to constant weight at 85°C. The algae were left to grow for 5-7 days after which they were harvested, dried to constant weight and analyzed for final tissue N or P content. Growth rates were estimated from changes in biomass over time assuming exponential growth:

$$\mu = \frac{\ln B_T - \ln B_0}{t} \quad \text{Eq. 3}$$

where μ is the relative growth rate (d⁻¹), B_0 and B_T are the initial and final DW biomass and t is the incubation time. Growth rates were plotted against tissue nutrient concentrations and the Droop equation was fitted to data using least square, non-linear regression (SYSTAT v. 13):

$$\mu = \mu_{max} \times \left(1 - \frac{Q_s}{Q}\right) \quad \text{Eq. 4}$$

where μ is the relative growth rate (d⁻¹), μ_{max} is the maximum growth rate, Q_s is the subsistence cell quota (in % of DW), i.e. the lowest tissue nutrient concentration that allows growth and Q is the average tissue N or P concentration (i.e. = $(Q_{init} + Q_{final})/2$). The critical cell quota (Q_C) was defined as the tissue nutrient concentration above which growth is not limited by lack of nutrients. The Droop function is a continuous function so we arbitrarily determined Q_C as the tissue nutrient concentration where the corresponding growth rate equaled 67% of the estimated μ_{max} (Pedersen and Borum 1996).

Modeling substrate dependent growth. The kinetics of substrate dependent growth were estimated by combining data for substrate dependent uptake kinetics and tissue nutrient dependent growth. Substrate dependent growth of macroalgae is often described by Monod kinetics (e.g. Rosenberg et al. 1984) with parameters μ^*_{max} and K_μ , which can be estimated

from equations 5 and 6 assuming that nutrient uptake and use of nutrients for growth is in steady state (Turpin 1988):

$$\mu_{max}^* = \frac{(\mu_{max} \times V_{max})}{[(\mu_{max} \times Q_S) + V_{max}]} \quad \text{Eq. 5}$$

$$K_{\mu} = \frac{(K_m \times \mu_{max} \times Q_{min})}{[(\mu_{max} \times Q_S) + V_{max}]} \quad \text{Eq. 6}$$

where V_{max} and K_m are the maximum uptake rate (in $\mu \text{ mol g}^{-1} \text{ DW h}^{-1}$) and the Michaelis-Menten constant (in μM), respectively, obtained from the nutrient uptake experiments (Eq. 2) while μ_{max} and Q_S are the maximum growth rate (d^{-1}) and subsistence cell quota (in % DW) obtained from the growth experiments (Eq. 4).

Storage capacity. The amount of N or P being stored in excess of what is needed to obtain maximum growth was estimated as the difference between the critical quota (Q_C) and the highest observed quota (Q_{max}) in algae from the growth experiment. The excess amount of cellular N or P, respectively, can support growth at maximum rates until Q_C is reached after which growth ceases as the cell quota approaches Q_S . We defined storage capacity as the number of days ($T_{Storage}$) that this pool of N or P could support growth (at maximum and reduced rates) without additional acquisition of N or P from the medium. The storage capacity was estimated from a numerical solution of equation 4 for Q . We used a time step of 0.1 day for each iteration and started out with an initial cell quota equal to the observed Q_{max} . A new cell quota was estimated for each time step using the estimated growth rate from the previous time step. The storage capacity that could support non-nutrient limited growth was then defined as the time passing until the cell quota reached Q_C while the storage capacity that could support growth at reduced rates was defined as the time it took to reduce the quota from Q_C to Q_S . Potential time lags to mobilize nutrient reserves were not accounted for.

Decomposition and mineralization. Decomposition of *G. vermiculophylla* and mineralization of N and P bound in its tissue was studied using litterbags. 105 algal samples

(each 0.6 – 2.5 g FW) were cleaned and their initial FW biomass determined. Fifteen samples were initially sacrificed and dried for determination of the initial DW:FW ratio. The remaining ninety samples were placed in separate litterbags with a mesh size of 1 mm, which were distributed equally among nine aquaria. Each aquarium had a volume of 45 L and was filled with ca. 20 L of sediment from the sampling site and 20 L of seawater (salinity 25). The aquaria were kept dark in 3 climate chambers with temperatures 5, 15 and 25°C, respectively, thus simulating a typical winter, spring/fall or summer situation. The water was bubbled with atmospheric air to keep it aerated and exchanged with freshly collected seawater monthly. The litterbags with live algae were covered by ca. 1 cm of sediment in an attempt to simulate slight burial as observed in the field. Three litterbags (one per replicate aquarium at each temperature) were retrieved periodically, and the algal remains were rinsed for sand and mud, dried to constant DW at 85°C, weighed and stored for later analysis of tissue nutrients (C, N and P). Decomposition rates were estimated by fitting a multiple-G model (Eq.; Westrich and Berner 1984) to the data (i.e. remaining biomass vs. time). This model assumes that detritus may be made up by several fractions, each decomposing at a specific rate (k_i) plus a refractory fraction (G_R) that does not decompose within the time-scale studied. If only one actively decomposing pool of matter and a refractory pool can be deduced from data, the model simplifies to:

$$B_T = B_0 e^{-kt} + G_R \quad \text{Eq. 7}$$

where B_T and B_0 are the final and initial biomass (measured in units of C, N or P), k is the decay rate, t is the number of days elapsed since initiating the experiment and G_R is the size of the residual (i.e. non-reactive) fraction of the detritus.

Tissue nutrient analyses. Tissue concentrations of C and N were determined on dried and ground samples using an EA 1110 CHNS elemental analyzer (CE Instruments). Tissue P was determined on dried and ground algae after oxidation with boiling H_2SO_4 followed by

spectrophotometric analysis (Strickland and Parsons 1968).

Results

Nutrient uptake kinetics. Uptake rates of NH_4^+ , NO_3^- and PO_4^{3-} increased with increasing substrate concentration and saturated at high concentrations (figure 1) why uptake kinetics were described by the Michaelis-Menten function (R^2 -values ranged from 0.629 to 0.930; all p-values <0.001). The maximum surge uptake rate of NH_4^+ was 2-fold higher than the maximum assimilation rate, while the half-saturation constant for uptake (K_m) during surge uptake was 3-fold higher than that for assimilation. These differences resulted in a higher affinity (α) for NH_4^+ at low substrate concentrations during assimilation than during surge uptake. The initial maximum uptake rate for NO_3^- was not different from that observed later in the experiment (i.e. no surge uptake), but it was 3 to 4-fold lower than both V_{max}^{Surge} and V_{max}^{Ass} for NH_4^+ . K_m for NO_3^- averaged 9 μM and the affinity at low substrate concentration ca. 1.7 μM . Initial V_{max} and K_m for PO_4^{3-} were ca. 2-fold higher than for P-uptake occurring after 30 minutes whereas the affinity for P at low PO_4^{3-} concentrations (α) was approximately the same for surge uptake and assimilation.

Growth rate and nutrient requirements. Growth rate increased as a function of N or P quotas (figure 2; R^2 for N = 0.876, $p < 0.001$; R^2 for P = 0.876, $p < 0.001$). Estimated maximum growth rate at infinite N or P quota varied slightly between the two experiments (0.088 – 0.105 d^{-1}), but did not differ significantly according to the 95% CL's (table 2). The N subsistence quota (Q_S) was 0.71% of DW while the critical N quota (Q_C) was 2.14% of DW, resulting in a N-requirement of 169 $\mu\text{mol N g}^{-1} \text{DW d}^{-1}$ at maximum growth rate. The subsistence quota for P was 0.05% of DW while the critical P quota was 0.14% of DW, resulting in a P-demand of 4.1 $\mu\text{mol P g}^{-1} \text{DW d}^{-1}$.

Substrate dependent growth. Substrate dependent growth was modeled from data on uptake kinetics and quota dependent growth and was represented by the derived Monod parameters (table 3). Estimated maximum growth rate based on uptake of NH_4^+ (0.098 d^{-1}) or NO_3^- (0.091 d^{-1}) as the N source did not differ much. In contrast, the half-saturation constant for growth (K_μ) based on NO_3^- was 2-fold larger than that for growth on NH_4^+ indicating a higher affinity for NH_4^+ . Substrate concentrations needed to saturate growth (S_{Sat}) ranged from $5 \text{ }\mu\text{M}$ for NH_4^+ to $10 \text{ }\mu\text{M}$ for NO_3^- . Estimated maximum growth rate based on uptake of PO_4^{3-} (0.084 d^{-1}) was slightly lower than for NH_4^+ or NO_3^- and K_μ was $0.12 \text{ }\mu\text{M PO}_4^{3-}$. The substrate concentration needed to support maximum growth rate was ca. $1 \text{ }\mu\text{M PO}_4^{3-}$.

Nutrient storage capacity. The amount of N stored in excess of the critical quota (i.e. $Q_{\text{max}} - Q_C$) attained $14.0 \text{ mg N g}^{-1} \text{ DW}$, while the internal quantity of N that could support sustained growth at reduced rates (i.e. $Q_C - Q_S$) was $14.3 \text{ mg N g}^{-1} \text{ DW}$ (table 2). These internal stocks of N could support continual growth at maximum or reduced rates for a total of 33 days (6.5 days at maximum rate and 26.6 days at reduced rates) without acquisition of dissolved N from the surroundings (table 4). The amount of P in excess of the critical limit was $1.3 \text{ mg P g}^{-1} \text{ DW}$ and the amount of P that could support sustained growth at reduced rates was $1.0 \text{ mg P g}^{-1} \text{ DW}$ (table 2). The internal stocks of P were could support growth for a total period of 43 days (10.5 days at maximum rate and 32.5 days at reduced rates; table 4) without having to acquire dissolved P from the medium.

Decomposition and mineralization. Decomposition of *G. vermiculophylla* followed a simple 1-G model without a significant refractory pool (i.e. a simple exponential decline; figure 3, table 5; R^2 ranged from 0.774 to 0.908, all p-values <0.001) since this model provided better fits (higher R^2 -values) than multiple G-models with or without a refractory pool. All biomass disappeared within 120 days in the 15 and 25°C treatments, while about 30% of the original biomass was left in the 5°C treatment at the end of the experiment. Decay rates based on loss

of C biomass ranged from 0.013 to 0.065 d⁻¹ depending on temperature and increased almost 5-fold across the temperature range from 5 to 25°C. Temperature had the same effect on loss of tissue-bound N and P. Decomposition rates ranged from 0.016 to 0.079 d⁻¹ when expressed in units of N and were marginally higher than when expressed in units of C. Decay rates expressed in units of P ranged from 0.023 to 0.1 d⁻¹ depending on temperature and were substantially higher than those expressed in units of C or N, indicating that P was mineralized faster than C and N during decomposition.

Discussion

The balance between uptake capacity and nutrient requirements and, thus, the risk of suffering nutrient limitation during periods of low nutrient availability is related to the maximum growth rate of algae. Fast-growing algae have a large relative surface area (i.e. high SA:V ratio) and a high capacity for nutrient uptake (Wallentinus 1984; Hein et al. 1995), but also large nutrient requirements per unit biomass and time dictated by their fast growth (Pedersen and Borum 1997; Pedersen et al. 2010). In contrast, slow-growing species have lower uptake capacities and lower requirements, but uptake rate and nutrient demands are better scaled at low nutrient availability in these algae (Pedersen and Borum 1997; Pedersen et al. 2010), why slow-growing algae generally can grow at near maximum rates when concentrations of dissolved inorganic N (DIN) and P (DIP) in the water are low.

Our findings failed to support the hypothesis presented by Nejrup and Pedersen (2010); *G. vermiculophylla* did not have an exceptionally high affinity for inorganic nutrients relative to its requirements for growth. Nutrient demands are largely determined by apparent growth rate and critical cell quotas. *G. vermiculophylla* attained maximum growth rates between 0.09 and 0.11 d⁻¹, which is comparable to those reported from other studies (Yokoya et al. 1999; Raikar et al. 2001; Nejrup and Pedersen 2010; Nejrup et al. 2013), but lower than

for truly fast-growing, sheet-like and filamentous species and higher than for most fucoids and Laminarians (see table 6 for a comparison with algae indigenous to Scandinavian waters). *G. vermiculophylla* can thus be ranked as an algal species with an intermediate growth rate.

The experimentally determined critical N quota of *G. vermiculophylla* is rather high and comparable to those of fast-growing species such as *U. lactuca* and *C. virgatum* (table 6). Red algae have generally higher N quotas than green and brown algae due to the prevalence of protein rich pigments in red algae (Hurd et al. 2014). The critical P quota of *G. vermiculophylla* was, in contrast, low and corresponded to those of more slow-growing species such as *F. vesiculosus* and *A. nodosum* (table 6). The observed maximum growth rates and critical N and P quotas resulted in maximum N and P requirements corresponding to ca. 169 and 4 $\mu\text{mol N or P g}^{-1} \text{ DW d}^{-1}$, respectively, which is lower than for truly fast-growing algae such as *U. lactuca* and *C. virgatum*, but higher than those for more slow-growing species (Pedersen and Borum 1997; Pedersen et al. 2010). High nutrient requirements do not necessarily represent a problem if the affinity for these nutrients is high enough. The obtained nutrient uptake kinetics of *G. vermiculophylla* corresponded to those found by other authors (e.g. Tyler et al. 2005; Abreu et al. 2011) and show that maximum uptake rates for dissolved inorganic N and P by far exceed the requirements even when *G. vermiculophylla* is growing at maximum rate. Such uptake rates can, however, only be obtained at relatively high and often ecologically irrelevant substrate concentrations. The main question was therefore whether the capacity of *G. vermiculophylla* to acquire nutrients is better scaled to growth related nutrient demands than in native algae that it potentially competes with. This is best evaluated by comparing species-specific substrate dependent growth and its related parameters across species. The half-saturation constants (K_{μ}) for growth and the substrate concentrations needed to saturate growth (S_{SAT}) on either NH_4^+ or NO_3^- tended to be higher than those of more slow-growing algae and

comparable to those of faster growing algae showing that *G. vermiculophylla* requires relatively high DIN concentrations to saturate growth while the opposite is true for DIP. The half-saturation constant (K_{μ}) for growth on PO_4^{3-} and the concentration of DIP needed to saturate growth were low and comparable to those of more slow-growing algae such as *Fucus vesiculosus* and *F. serratus*, which could give *G. vermiculophylla* an advantage under low P availability. P-limitation is however rare in most temperate estuaries with terrigenous sediments, where N-limitation is more common (e.g. Howarth and Marino 2006). Nejrup and Pedersen (2010) showed that the tissue N:P ratio in *G. vermiculophylla* from Holckenhavn Fjord and Fyns Hoved reached 40-45 for a short period in early spring, thus inferring P-limitation. The absolute P-content in these algae was however relatively high (>0.2 % of DW) and experimental fertilization did not stimulate growth. Slow growth in early spring seems thus to be dictated by low water temperature and insolation rather than by P-limitation and low P-requirements do therefore not seem to represent a clear advantage for *G. vermiculophylla*. In summary, we found no clear evidence that the nutrient kinetics of *G. vermiculophylla* result in higher nutrient use efficiency than in comparable, sympatric indigenous algal species and therefore, no indication that *G. vermiculophylla* should have a specific advantage relative to native species under nutrient replete conditions.

Nutrient storage plays an essential role for seaweeds in areas where nutrient availability undergoes large seasonal variations. Little is known about the forms in which excess P is stored in macroalgae, but N is mainly stored as proteins and amino acids while inorganic N and N bound in pigments may constitute smaller and less important N reserves (Bird et al. 1982; McGlathery et al. 1996). Although some amino acids play essential roles for osmoregulation (e.g. proline; Kirst 1989) and photoprotection (e.g. mycosporine-like amino acids; Karsten et al. 2000) there is a strong correlation between total N content and the pool sizes of proteins, amino acids, inorganic N and pigment bound N, which shows that all these

pools take part in N storage and can be mobilized when necessary (Bird et al. 1982; McGlathery et al. 1996).

The amount of N and P stored in excess of that needed to support the maximum growth rate in *G. vermiculophylla* was ca. 14 mg N g⁻¹ DW and 1.3 mg P g⁻¹ DW, respectively. The observed N-reserve was somewhat less than for a range of indigenous algal species (Pedersen and Borum 1996; Pedersen et al. 2010), which suggests that the algae used in our experiments may not have been saturated with nutrients. The storage capacity for nutrients (defined as the time where growth could be sustained without additional nutrient uptake from the medium) was ca. 33 days for N and 43 days for P, respectively. The storage capacity for N was larger for a number of fast-growing indigenous algae (*U. lactuca*, *C. virgatum*, *Cladophora* sp. and *Chaetomorpha linum*: range from 9.6 to 17.5 days), but smaller than for slow-growing *F. vesiculosus* (45.9 days; Pedersen and Borum 1996).

The N and P-reserves obtained through winter and spring can obviously not sustain maximum growth of *G. vermiculophylla* throughout extended periods of low nutrient availability (i.e. mid May to mid September). However, even though *in situ* nutrient concentrations in Holckenhavn Fjord and Fyns Hoved were low (Nejrup and Pedersen 2010), they were not low enough to prevent nutrient uptake from the water completely. The observed concentrations of DIN ranged between 1 and 3 µM from mid May to mid September and should be high enough to cover about 50% of the N-demand for maximum growth, meaning that the N reserves could last for about twice as long as estimated assuming no uptake (i.e. ca. 2 months). Equally important, average *in situ* growth rates in Holckenhavn Fjord during the growing season were some what lower than those attained in this study (ca. 0.05 d⁻¹, range 0.032 – 0.065 d⁻¹; Nejrup and Pedersen 2010), meaning that ‘dilution’ of the internal nutrient reserves would occur more slowly and the stores last longer than predicted when assuming growth at maximum attainable rates. In other words, the combined effect of continuous

nutrient uptake (albeit at reduced rates) and slower growth (i.e. lower nutrient demands per unit biomass and time) increases the storage capacity of the algae by a factor of ca. 4, i.e. to 130-160 days, which should be enough to sustain non-nutrient limited growth during most of the major growth season. This conclusion is supported by the fact that Nejrup and Pedersen (2010) were unable to stimulate *in situ* growth significantly by experimental nutrient enrichment and by the fact that the N content in algae from the control treatment in Holckenhavn Fjord and Fyns Hoved remained close to ca. 2.5% of DW (i.e. above the critical N content) throughout summer. Relatively fast and non-nutrient limited growth by *G. vermiculophylla* during summer can thus be explained by the use of reserve N (and P) rather than by an extraordinarily high affinity for inorganic nutrients.

The large biomass of *G. vermiculophylla* found in some estuaries, e.g. >1000 g DW m⁻² in some samples from Hog Island Bay, Virginia, USA (Thomsen et al. 2006), 464 g DW m⁻² on average in Holckenhavn Fjord, Denmark (Nejrup and Pedersen 2010) and 6-700 g DW m⁻² in parts of Venice lagoon, Italy (Sfriso et al. 2012) suggests that large amounts of DIN and DIP are assimilated and become bound in living or dead biomass and, thus, that *G. vermiculophylla* has the potential to affect nutrient cycling significantly in such systems (e.g. Tyler et al. 2003; Tyler and McGlathery 2006). Like for other bloom-forming algae, the biomass of *G. vermiculophylla* might undergo large and quick temporal variations where algae suffer high mortality and the biomass is turned into detritus that decomposes under release of nutrients. Whether detritus originating from *G. vermiculophylla* serves as a temporary sink for nutrients during summer or functions as an internal source of nutrients within the system depends on: 1) the turn-over of biomass and, thus, the production of detritus and, 2) the rate at which that detritus decays and the bound nutrients become mineralized.

We have no direct estimates of the biomass turnover of *G. vermiculophylla*, but data on seasonal changes in biomass and *in situ* growth rates from Holckenhavn Fjord, from

Nejrup and Pedersen (2010) show that the potential production of *G. vermiculophylla* may reach ca. 5200 g DW m⁻² during the growing season (from March to October) corresponding to a net incorporation of ca. 78 g N and ca. 10 g P m⁻² (assuming a mean N and P content of 1.5% of DW and 0.2% of DW, respectively). The average load of total nitrogen (TN) and total phosphorus (TP) per unit of estuarine area across 47 Danish estuaries is ca. 20 g TN m⁻² y⁻¹ and 0.8 g TP m⁻² y⁻¹ (Conley et al. 2000) so *G. vermiculophylla* may have a substantial effect on nutrient cycling in Holckenhavn Fjord. The biomass of *G. vermiculophylla* changed little over the season (ranging from 464 g DW m⁻² in March to 176 g DW m⁻² in October), so biomass losses and, hence, the production of detritus must have been in the same order of magnitude as the production of biomass (estimated to ca. 5400 g DW m⁻² in 7 months) since grazing on *G. vermiculophylla* is insignificant (Nejrup and Pedersen 2010). Large amounts of nutrient are thus being acquired and bound into living and dead algal biomass and will become released when the detritus decomposes. Decomposition rates of *G. vermiculophylla* ranged from 0.013 to 0.065 d⁻¹ depending on temperature and were markedly lower than those reported by Conover et al. (2016). Conover et al. (2016) used dead tissues killed by freezing which were placed on top of the sediment (i.e. under aerobic conditions) whereas we used live material that was slightly covered by sediment (i.e. partly hypoxic conditions). Freezing may break the cell membrane and wall and cause a rapid loss of soluble compounds from the detritus and, thus, speed up decomposition. The use of live material may, in contrast, delay initial decay and the initial loss of biomass may be due to respiration rather than decay. Dark respiration rate in *G. vermiculophylla* corresponds, however, to a much smaller biomass loss than that observed in our decomposition study (e.g. <0.003 d⁻¹; Nejrup et al. 2013) suggesting that the biomass losses we observed were caused by decomposition mainly.

Decomposition of *G. vermiculophylla* was strongly temperature dependent; decomposition was 4-5 fold faster at 25°C than at 5°C, corresponding to a half-time of about

10 days under late summer conditions suggesting that nutrients bound in detritus generated during summer and early autumn are released almost completely within the time-scale of weeks whereas it takes much longer during winter and early spring. An increasing algal biomass in early spring and subsequent production of detritus during early summer may thus immobilize bio-available nutrients whereas the opposite will be true in late summer and early autumn where the production of detritus increases and dead *G. vermiculophylla* decomposes fast and, thus, will act as a major source of nutrients. Nutrients released from living and dead *G. vermiculophylla* may not only be recycled back to live and nutrient deplete *Gracilaria* in the upper layers of the mats, but may also be taken up by other primary producers including phytoplankton and microphytobenthos or diffuse into the sediment where it can take part in biogeochemical processes (Hardison et al. 2010; Gulbransen and McGlathery 2013).

Decomposition rate for *G. vermiculophylla* at 15°C were obtained under the same conditions as rates for other estuarine macroalgae reported by Banta et al. (2004) and Pedersen et al. (2005), which allows for a direct comparison with these data. Detritus from *G. vermiculophylla* decomposed completely within 120 days at 15°C. The decay rate (0.032 d^{-1}) was lower than those of *Ulva lactuca* and *Ceramium virgatum* ($0.038 - 0.040\text{ d}^{-1}$) and similar to that of *Fucus vesiculosus* (0.028 d^{-1} ; Banta et al. 2004). *F. vesiculosus* contains however refractory compounds, which will leave ca. 7% of the initial biomass after 340 days (Banta et al. 2004). Detritus from *G. vermiculophylla* decomposed markedly faster than that from *Sargassum muticum* (0.016 d^{-1}) and *Halidrys siliquosa* (0.019 d^{-1}), respectively (Pedersen et al. 2005). The release of nutrients through decomposition and, thus, the potential impact of *G. vermiculophylla* on local nutrient cycling depends therefore partly on which species it has succeeded. Nutrient cycling may be slowed down when fast-growing species like *Ulva* sp. and *Ceramium* sp. are replaced by *Gracilaria* because the former are grazed more and their detritus decomposes faster than for *G. vermiculophylla*. Dominance by *G. vermiculophylla*

496 will thus tend cause a slower turnover of nutrients and the biomass of living and dead
497 *Gracilaria* may act as a temporary sink for nutrients. The opposite is expected to be the case
498 if *G. vermiculophylla* succeeds slow-growing species such as *Fucus* sp. *Halidrys siliquosa*
499 etc., which are less susceptible to grazing and produces detritus that decomposes slowly
500 and/or incompletely. Here dominance of *G. vermiculophylla* will tend to speed up the
501 turnover of nutrients due to its relatively fast and complete decomposition.

502

503 **References**

- 504 Abreu MH, Pereira R, Buschmann AH, Sousa-Pinto I, Yarish C (2011) Nitrogen uptake
505 responses of *Gracilaria vermiculophylla* (Ohmi) Papenfuss under combined and single
506 addition of nitrate and ammonium. J Exp Mar Biol Ecol 407: 190–199.
- 507 Banta GT, Pedersen MF, Nielsen SL (2004) Decomposition of marine primary producers:
508 Consequences for nutrient recycling and retention in coastal ecosystems. In: GT Banta,
509 MF Pedersen, SL Nielsen (eds.), Estuarine nutrient cycling: The influence of primary
510 producers: The fate of nutrients and biomass (pp. 187-216). Kluwer Academic Publishers,
511 Dordrecht/Boston/London.
- 512 Bird KT, Habig C, DeBusk T (1982) Nitrogen allocation and storage patterns in *Gracilaria*
513 *tikvahiae* (Rhodophyta). J Phycol 18: 344-348.
- 514 Bokn TL, Duarte CM, Pedersen MF, Marba N, Moy FE, Barrón C, Bjerkeng B, Borum J,
515 Christie H, Fotel FL, Hoell EE, Karez R, Kersting K, Kraufvelin P, Lindblad C, Olsen M,
516 Sommer U, Sørensen K (2003) The response of experimental rocky shore communities to
517 nutrient additions: A synthesis of the EULIT experiment. Ecosystems 6: 577-594.
- 518 Bower CE, Holm-Hansen T (1980) A salicylate-hypochlorite method for determining
519 ammonia in seawater. Can J Fish Aquat Sci 37: 794-798.
- 520 Buchsbaum R, Valiela I, Swain T, Dzierzeski M, Allen S (1991) Available and refractory
521 nitrogen in detritus of coastal vascular plants and macroalgae. Mar Ecol Prog Ser 72: 131–
522 143.
- 523 Byers JE, Gribben PE, Yeager C, Sotka EE (2012) Impacts of an abundant introduced
524 ecosystem engineer within mudflats in the southeastern US coast. Biol Invasions 14:
525 2587- 2600.
- 526 Conley DJ, Kaas H, Møhlenberg F, Rasmussen B, Windolf J (2000) Characteristics of Danish
527 Estuaries. Estuaries 23: 820-837.

528 Duarte CM, Cebrian J (1996) The fate of marine autotrophic production. *Limnol Oceanogr*
 529 41: 1758-1766.

530 Enriquez S, Duarte CM, Sand-Jensen K (1993) Patterns in decomposition rates among
 531 photosynthetic organisms: the importance of detritus C:N:P content. *Oecologia* 94: 457–
 532 471.

533 Gulbransen D, McGlathery K (2013) Nitrogen transfers mediated by perennial, non-native
 534 macroalga: a ^{15}N tracer study. *Mar Ecol Prog Ser* 482: 299-304.

535 Hammann M, Wang G, Rickert E, Boo SM, Weinberger F (2013) Invasion success of the
 536 seaweed *Gracilaria vermiculophylla* correlates with low palatability. *Mar Ecol Prog Ser*
 537 486: 93-103.

538 Hammann M, Rempt M, Pohnert G, Wang G, Boo SM, Weinberger F (2016) Increased
 539 potential for wound activated production of Prostaglandin E-2 and related toxic
 540 compounds in non-native populations of *Gracilaria vermiculophylla*. *Harmful Algae* 51:
 541 81-88.

542 Hardison A, Canuel E, Anderson I, Veuger B (2010) Fate of macroalgae in benthic systems:
 543 carbon and nitrogen cycling within the microbial community. *Mar Ecol Prog Ser* 414: 41–
 544 55.

545 Harrison PJ, Parslow JS, Conway HL (1989) Determination of nutrient uptake kinetic
 546 parameters: a comparison of methods. *Mar Ecol Prog Ser* 52: 301-312.

547 Healey FP (1980) Slope of the Monod equation as an indicator of advantage in nutrient
 548 competition. *Microb Ecol* 5: 281-286.

549 Hein M, Pedersen MF, Sand-Jensen K (1995). Size-dependent nitrogen uptake in micro-and
 550 macroalgae. *Mar Ecol Prog Ser* 118: 247-253.

551 Howarth RW, Marino R (2006) Nitrogen is the limiting nutrient for eutrophication in coastal
 552 marine ecosystems: evolving views over three decades. *Limnol Oceanogr* 51: 364–376.

553 Hurd CL, Harrison PJ, Bischof K, Lobban CS (2014) Seaweed ecology and physiology.
 554 Cambridge University Press. Cambridge, UK.

555 Karez R, Engelbert S, Kraufvelin P, Pedersen MF, Sommer U (2004) Biomass response and
 556 changes in composition of ephemeral macroalgal assemblages along an experimental
 557 gradient of nutrient enrichment. *Aquat Bot* 78: 103-117.

558 Karsten U, Sawall T, West J, Wiencke C (2000) Ultraviolet sunscreen compounds in
 559 epiphytic red algae from mangroves. *Hydrobiologia* 432: 159-171.

560 Kirst GO (1989) Salinity tolerance of eukaryotic marine algae. *Annu Rev Plant Phys* 41: 21–
 561 53.

562 Kraufvelin P, Moy FE, Christie H, Bokn TL (2006) Nutrient addition to experimental rocky
 563 shore communities revisited: delayed responses, rapid recovery. *Ecosystems* 9: 1076–
 564 1093.

565 Kraufvelin P, Lindholm A, Pedersen MF, Kirkerud LA, Bonsdorff E (2010) Biomass,
 566 diversity and production of rocky shore macroalgae at two nutrient enrichment and wave
 567 action levels. *Mar Biol* 157: 29-47.

568 McGlathery KJ, Pedersen MF, Borum J (1996) Changes in intracellular nitrogen pools and
 569 feedback controls on nitrogen uptake in *Chaetomorpha linum* (Chlorophyta). *J Phycol* 32:
 570 393-401.

571 Mollet J, Rahaoui A, Lemoine Y (1998) Yield, chemical composition and gel strength of
 572 agarocolloids of *Gracilaria gracilis*, *Gracilariopsis longissima* and the newly reported
 573 *Gracilaria cf. vermiculophylla* from Roscoff (Brittany, France). *J Appl Phycol* 10: 59–66.

574 Nejrup LB, Pedersen MF (2010) Growth and biomass development of the introduced red alga
 575 *Gracilaria vermiculophylla* is unaffected by nutrient limitation and grazing. *Aquat Biol*
 576 10: 249-259.

577 Nejrup LB, Pedersen MF (2012) The effect of temporal variability in salinity on the invasive
 578 red alga *Gracilaria vermiculophylla*. *Europ J Phycol* 47: 254-263.

579 Nejrup LB, Pedersen MF, Vinzent J (2012) Grazer avoidance may explain the invasiveness of
 580 the red alga *Gracilaria vermiculophylla* in Scandinavian waters. *Mar Biol* 159: 1703–
 581 1712.

582 Nejrup LB, Staehr PA, Thomsen MS (2013) Temperature- and light-dependent growth and
 583 metabolism of the invasive red algae *Gracilaria vermiculophylla* – a comparison with two
 584 native macroalgae. *Europ J Phycol* 48: 295–308.

585 Nyberg CD, Thomsen MS, Wallentinus I (2009) Flora and fauna associated with the
 586 introduced red alga *Gracilaria vermiculophylla*. *Europ J Phycol* 44: 395–403.

587 Nylund GM, Weinberger F, Rempt M, Pohnert G (2011) Metabolomic assessment of induced
 588 and activated chemical defence in the invasive red alga *Gracilaria vermiculophylla*. *PLoS*
 589 *One* 6: e29359.

590 Pedersen MF (1994) Transient ammonium uptake in the macroalga *Ulva lactuca*
 591 (Chlorophyta): Nature, regulation, and the consequences for choice of measuring
 592 technique. *J Phycol* 30: 980-986.

593 Pedersen MF, Borum J (1996) Nutrient control of algal growth in estuarine waters: Nutrient
 594 limitation and the importance of nitrogen requirements and nitrogen storage among
 595 phytoplankton and species of macroalgae. *Mar Ecol Prog Ser* 142: 261-272.

596 Pedersen MF, Borum J (1997) Nutrient control of estuarine macroalgae: growth strategy and
 597 the balance between nitrogen requirements and uptake. *Mar Ecol Prog Ser* 161: 155-163.

598 Pedersen MF, Nielsen SL, Banta G (2004) Interactions between vegetation and nutrient
 599 dynamics in coastal marine ecosystems: an introduction. In: GT Banta, MF Pedersen, SL
 600 Nielsen (eds.), *Estuarine nutrient cycling: The influence of primary producers: The fate of*
 601 *nutrients and biomass* (pp. 1-15). Kluwer Academic Publishers, Dordrecht/Boston/London.

602 Pedersen MF, Borum J, Fotel FL (2010) Phosphorus dynamics and limitation of fast-and
 603 slow-growing temperate seaweeds in Oslofjord, Norway. *Mar Ecol Prog Ser* 399: 103-115.
 604 Pedersen MF, Staehr PA, Wernberg T, Thomsen MS (2005) Biomass dynamics of exotic
 605 *Sargassum muticum* and native *Halidrys siliquosa* in Limfjorden, Denmark-Implications
 606 of species replacements on turnover rates. *Aquat Bot* 83: 31-47.
 607 Raikar SV, Iima M, Fujita Y (2001) Effect of temperature, salinity and light intensity on the
 608 growth of *Gracilaria* spp. (Gracilariales, Rhodophyta) from Japan, Malaysia and India.
 609 *Indian J Mar Sci* 30: 98-104.
 610 Rosenberg G, Probyn TA, Mann KH (1984) Nutrient uptake and growth kinetics in brown
 611 seaweeds: Response to continuous and single additions of ammonium. *J Exp Mar Biol Ecol*
 612 80: 125-146.
 613 Rueness, J. (2005). Life history and molecular sequences of *Gracilaria vermiculophylla*
 614 (Gracilariales, Rhodophyta), a new introduction to European waters. *Phycologia* 44: 120-
 615 128.
 616 Sfriso A, Wolf MA, Maistro S, Sciuto K, Moro I (2012) Spreading and autoecology of the
 617 invasive species *Gracilaria vermiculophylla* (Gracilariales, Rhodophyta) in the lagoons of
 618 the north-western Adriatic Sea (Mediterranean Sea, Italy). *Est Coast Shelf Sci* 114: 192-
 619 198.
 620 Strickland JD, Parsons TR (1968) A practical handbook of seawater analysis. *Bull Fish Res*
 621 Board Can. No 167.
 622 Thomsen MS, McGlathery KJ, Tyler AC (2006) Macroalgal distribution in a shallow, soft
 623 bottom lagoon, with emphasis on the non-native *Gracilaria vermiculophylla* and *Codium*
 624 *fragile*. *Est Coast* 29: 465-473.
 625 Thomsen MS, Staehr PA, Nyberg CD, Schwærter S, Krause-Jensen D, Silliman BR (2007)
 626 *Gracilaria vermiculophylla* (Ohmi) Papenfuss, 1967 (Rhodophyta, Gracilariaceae) in

627 northern Europe, with emphasis on Danish conditions, and what to expect in the future.
 628 Aquat Invasions 2: 83-94.

629 Thomsen MS, McGlathery KJ (2007) Stress tolerance of the invasive macroalgae *Codium*
 630 *fragile* and *Gracilaria vermiculophylla* in a soft-bottom turbid lagoon. Biol Invasions 9:
 631 499–513.

632 Tseng CK, Xia BM (1999) On the *Gracilaria* in the Western Pacific and the Southeastern Asia
 633 Region. Bot Mar 42: 209-217.

634 Turpin DH (1988) Physiological mechanisms in phytoplankton resource competition. Chapter
 635 8 in Sandgren CD (ed) Growth and reproductive strategies of freshwater phytoplankton.
 636 Cambridge University Press. Cambridge, UK. pp. 316-368.

637 Tyler AC, McGlathery KJ, Macko SA (2005) Uptake of urea and amino acids by the
 638 macroalgae *Ulva lactuca* (Chlorophyta) and *Gracilaria vermiculophylla* (Rhodophyta).
 639 Mar Ecol Prog Ser 294: 161-172.

640 Tyler AC, McGlathery KJ (2003) Benthic algae control sediment-water column fluxes of
 641 organic and inorganic nitrogen compounds in a temperate lagoon. Limnol Oceanogr 48:
 642 2125-2137.

643 Tyler AC, McGlathery KJ (2006) Uptake and release of nitrogen by the macroalgae
 644 *Gracilaria vermiculophylla* (Rhodophyta). J Phycol 42: 515–525.

645 Wallentinus I (1984) Comparisons of nutrient uptake rates for Baltic macroalgae with
 646 different thallus morphologies. Mar Biol 80:215–225.

647 Wallentinus I, Nyberg CD (2007) Introduced marine organisms as habitat modifiers. Mar.
 648 Pollut Bull 55: 323–32.

649 Weinberger F, Buchholz B, Karez R, Wahl M (2008) The invasive red alga *Gracilaria*
 650 *vermiculophylla* in the Baltic Sea: adaptation to brackish water may compensate for light
 651 limitation. Aquat Biol 3: 251-264.

Westrich JT, Berner RA (1984) The role of sedimentary organic matter in bacterial sulfate reduction: The G model tested. *Limnol Oceanogr* 29: 236–249.

Yokoya NS, Kakita H, Obika H, Kitamura T (1999) Effects of environmental factors and plant growth regulators on growth of the red alga *Gracilaria vermiculophylla* from Shikoku Island, Japan. *Hydrobiologia*, 398/399, 339-347.

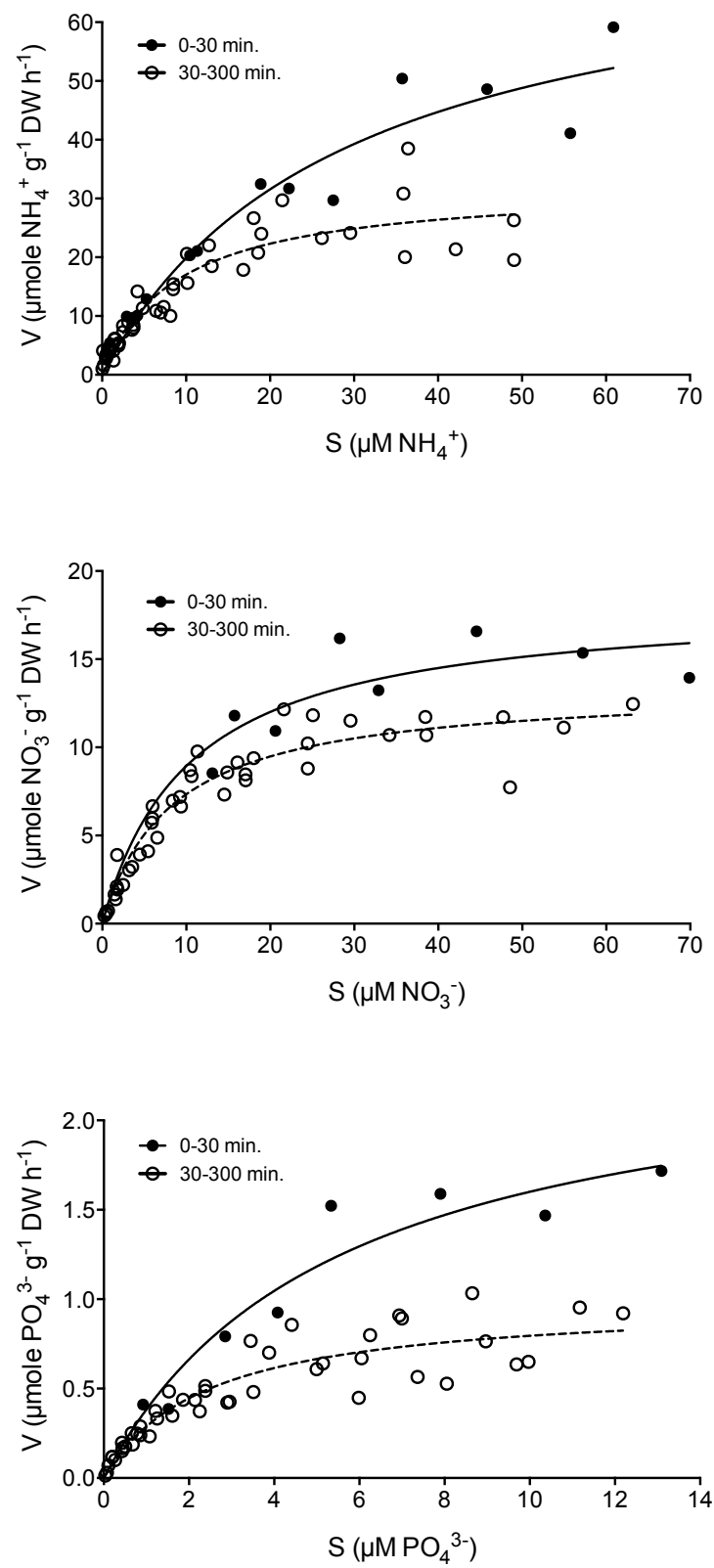
Figure legends

Fig. 1 *Gracilaria vermiculophylla*. Uptake rates (V) of ammonium, nitrate and phosphate as a function of substrate concentration (S). Surge uptake (●) was measured over the initial 30 minutes after exposure to nutrients, while assimilation rates (○) were measured later than 30 minutes after exposure. Lines represent the best fits of the Michalis-Menten function to the data

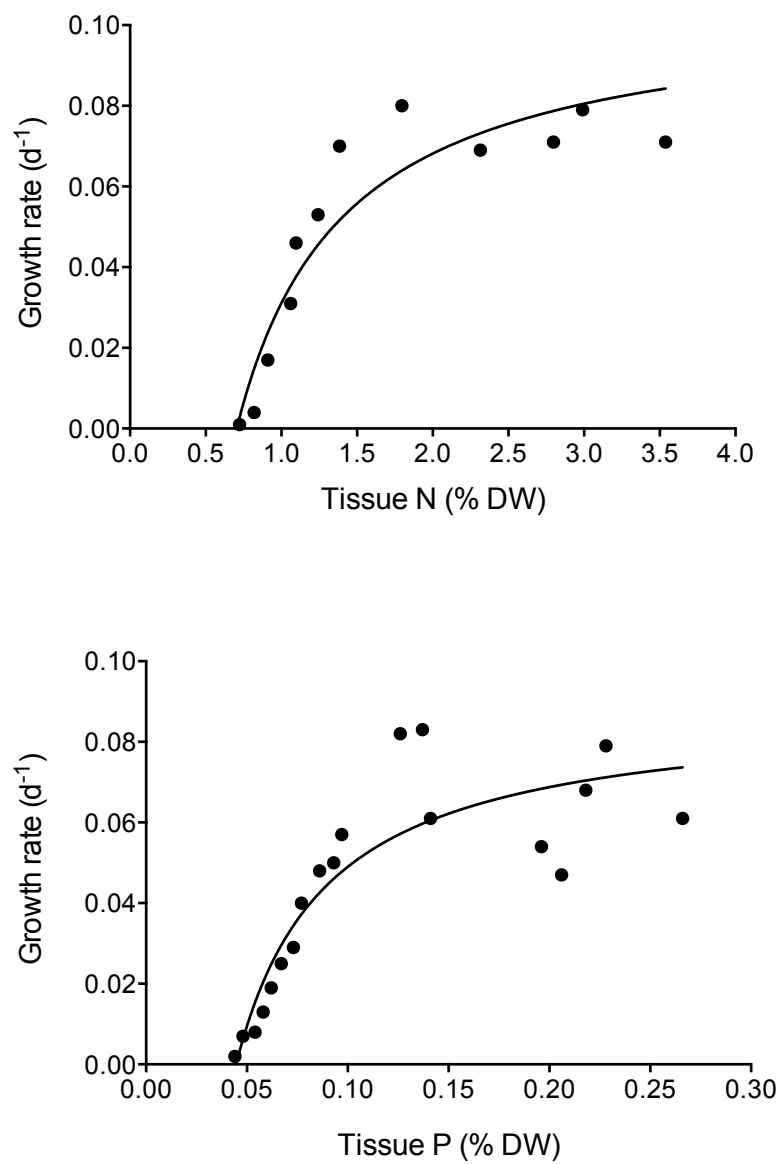
Fig. 2 *Gracilaria vermiculophylla*. Relationship between relative growth rate (μ) and N quota (figure A) or P quota (figure B) as determined in laboratory experiments. Lines represent the best fits of the Droop equation to the data

Fig. 3 *Gracilaria vermiculophylla*. Changes in C, N and P biomass during decomposition of dead *Gracilaria vermiculophylla* at three different temperatures. A: 5°C, B: 15°C and C: 25°C. Lines represent the best fits to an exponential function. Values are means \pm 95% confidence limits (n=3)

Figure 1.

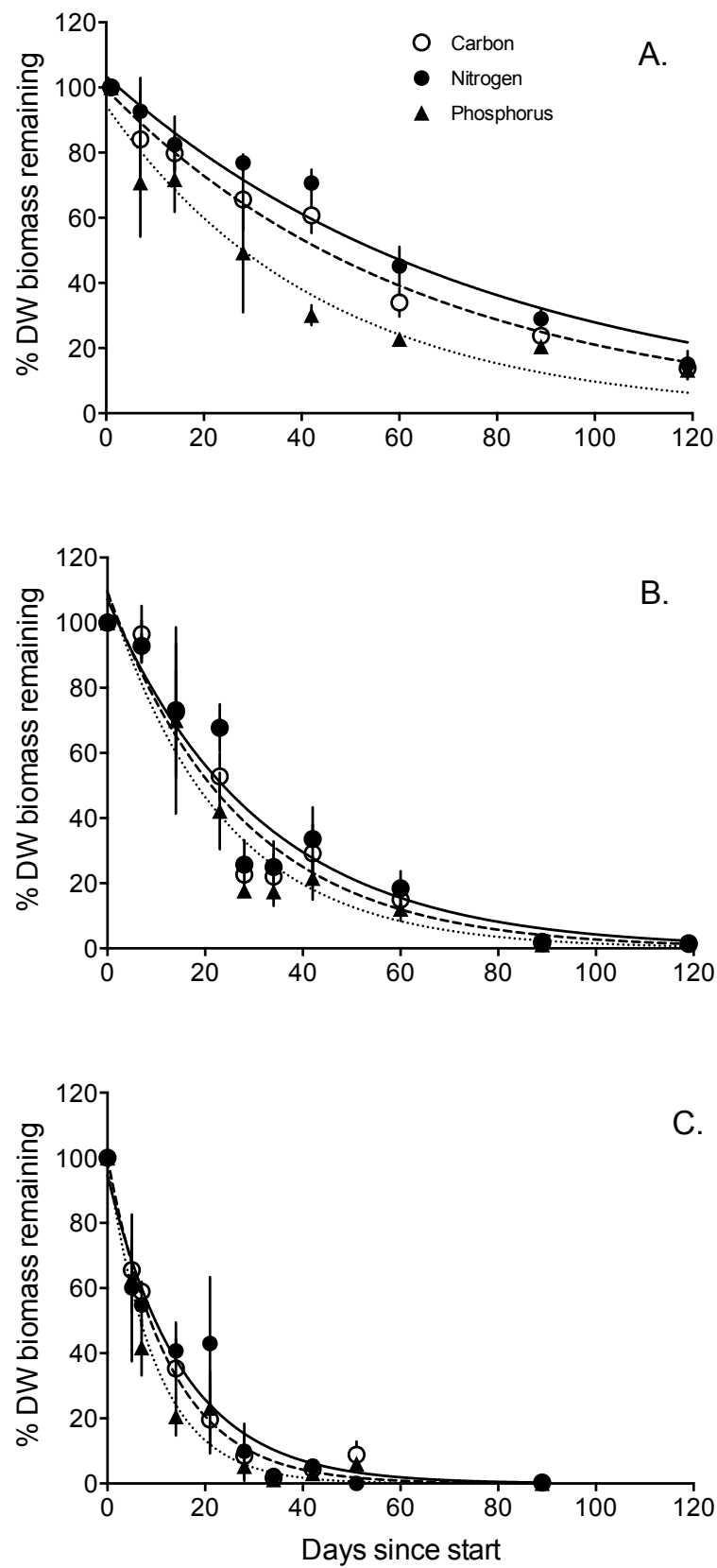


675 **Figure 2.**



676

677



680

Table 1. Nutrient (NH_4^+ , NO_3^- and PO_4^{3-}) uptake kinetics. Parameters V_{\max} ($\mu\text{mol N or P g}^{-1} \text{ DW h}^{-1}$), K_m (μM) and affinity for uptake at low substrate concentrations (V_{\max}/K_m ; Healy 1980) are given for both initial surge uptake measured over the first 30 minutes of exposure to nutrients and for uptake and assimilation measured later than 30 minutes after exposure to nutrients. Numbers are means $\pm 95\%$ confidence limits.

	Surge uptake (0-30 min.)			Assimilation (30-300 min.)		
	V_{\max} ($\mu\text{mol g}^{-1} \text{ DW h}^{-1}$)	K_m (μM)	V_{\max}/K_m	V_{\max} ($\mu\text{mol g}^{-1} \text{ DW h}^{-1}$)	K_m (μM)	V_{\max}/K_m
NH_4^+	76.8 ± 24.5	28.7 ± 19.7	2.68	32.3 ± 4.4	8.9 ± 3.2	3.63
NO_3^-	18.3 ± 5.6	10.4 ± 12.4	1.76	13.4 ± 1.3	8.3 ± 2.3	1.61
PO_4^{3-}	2.47 ± 1.01	5.5 ± 5.1	0.45	1.0 ± 0.2	2.4 ± 1.0	0.42

681

682

683

684

Table 2. Parameter estimates of maximum growth rate (μ_{\max}) and subsistence cell quota (Q_s) as determined from fitting the Droop function to data (i.e. growth rates vs. cell quota). Estimated means $\pm 95\%$ confidence limits. Critical cell quotas (Q_c) were estimated as the quota where growth rate equalled 66.7% of μ_{\max} while maximum cell quotas (Q_{\max}) correspond to the highest quotas obtained in the experiment.

	μ_{\max} (d^{-1})	Q_s (% of DW)	Q_c (% of DW)	Q_{\max} (% of DW)	Requirement ($\mu\text{mol g}^{-1} \text{ DW d}^{-1}$)
Nitrogen	0.105 ± 0.016	0.706 ± 0.098	2.14	3.54	169.2
Phosphorus	0.088 ± 0.013	0.045 ± 0.007	0.14	0.27	4.1

685

Table 3. Modelled substrate dependent growth kinetics. Monod parameters (μ_{max}^* and K_{μ}) and the substrate concentration at which 0.9 of the maximum growth rate is obtained (S_{sat}).

	μ_{max}^* (d ⁻¹)	K_{μ} (μ M)	μ_{max}/K_{μ}	S_{sat} (μ M)
Ammonium	0.098	0.57	0.17	5.1
Nitrate	0.091	1.17	0.08	10.4
Phosphorus	0.084	0.12	0.70	1.03

Table 4. Storage capacity ($T_{Storage}$) for N and P, respectively, in *Gracilaria vermiculophylla*.

	$T_{Storage}$ at max. growth rate (days)	$T_{Storage}$ at reduced growth rate (days)	$T_{Storage}$ total (days)
Storage capacity for N	6.5	26.5	33.0
Storage capacity for P	10.5	32.5	43.0

687
688
689
690
691
692
693
694
695

696

Table 5. Parameter estimates (Y_0 : intercept with Y-axis; k : decay rate and, $T_{0.5}$: half time) for decomposition of C biomass and mineralization of tissue bound N and P in *Gracilaria vermiculophylla*. Estimated means \pm 95% confidence limits. Decomposition experiments were conducted at 5°C (A), 15°C (B) and 25°C (C), respectively.

	Y_0 (%)	k (d ⁻¹)	$T_{0.5}$ (days)	R^2
C at 5°C	103.4 \pm 3.4	0.013 \pm 0.002	52.9 \pm 8.9	0.895
C at 15°C	107.2 \pm 14.0	0.032 \pm 0.007	21.5 \pm 4.1	0.857
C at 25°C	94.6 \pm 17.3	0.065 \pm 0.023	10.7 \pm 2.8	0.774
N at 5°C	99.5 \pm 9.5	0.016 \pm 0.004	44.6 \pm 7.9	0.877
N at 15°C	109.5 \pm 15.1	0.037 \pm 0.009	18.8 \pm 3.8	0.842
N at 25°C	99.9 \pm 9.8	0.079 \pm 0.015	12.7 \pm 5.3	0.926
P at 5°C	94.4 \pm 14.5	0.023 \pm 0.008	30.5 \pm 7.8	0.780
P at 15°C	109.8 \pm 17.8	0.043 \pm 0.012	16.2 \pm 3.7	0.805
P at 25°C	98.8 \pm 11.2	0.100 \pm 0.022	6.9 \pm 1.2	0.908

697

698

Table 6. Maximum relative growth rate (μ_{max}), critical N and P quotas (N_C and P_C), half-saturation constants for growth (K_μ) and saturating substrate concentrations (S_{sat}) for *Gracilaria vermiculophylla* and eleven common algal species native to Scandinavian waters.

Species	μ_{max} (d ⁻¹)	Critical quota		NH ₄ ⁺		NO ₃ ⁻		PO ₄ ³⁻	
		N _C	P _C	K _μ	S _{sat}	K _μ	S _{sat}	K _μ	S _{sat}
		(%DW)		(μM)		(μM)		(μM)	
<i>Ulva lactuca</i> ^{1,2,3}	0.196 - 0.452	2.17	0.20	0.75	6.71	1.45	13.02	0.27	2.39
<i>Ceramium virgatum</i> ^{1,2,3}	0.136 - 0.299	3.10	0.44	0.63	5.65	-	-	0.22	1.94
<i>Cladophora sp.</i> ^{1,2}	0.188 – 0.208	2.05	-	0.36	3.28	0.83	7.45	-	-
<i>Chaetomorpha linum</i> ^{1,2}	0.139 – 0.142	1.15	-	0.11	1.00	0.13	1.18	-	-
<i>Gracilaria vermiculophylla</i>	0.084 – 0.098	2.14	0.14	0.57	5.10	1.17	10.40	0.12	1.03
<i>Chordaria flagelliformis</i> ⁴	0.091 – 0.126			0.31					
<i>Codium fragile</i> ^{1,2}	0.074 – 0.083	1.58	-	0.25	2.22	0.57	5.11	-	-
<i>Fucus disticus</i> ⁴	0.067 – 0.081			0.24					
<i>Fucus vesiculosus</i> ^{1,2,3}	0.038 – 0.040	1.71	0.12	0.29	2.66	0.81	7.32	0.09	0.78
<i>Fucus serratus</i> ³	0.040	-	0.22	-	-	-	-	0.16	0.88
<i>Ascophyllum nodosum</i> ³	0.014	-	0.15	-	-	-	-	0.06	0.35
<i>Laminaria digitata</i> ³	0.006	-	0.22	-	-	-	-	0.03	0.15

Data sources:

¹⁾ Pedersen and Borum 1996

²⁾ Pedersen and Borum 1997

³⁾ Pedersen et al. 2010

⁴⁾ Rosenberg et al. 1984